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Editorial

Homage to Theodor Boveri (1862–1915): Boveri's Theory of Cancer as a Disease of the Chromosomes, and the Landscape of Genomic Imbalances in Human Carcinomas

During the session on "Genomic Instability and Cancer" at a recent meeting organized by the Center of Excellence in Chromosome Biology (CECB), held at the National Cancer Institute in Bethesda, MD, three of the four speakers referred to or acknowledged Theodor Boveri's (Fig. 1A) contributions to cancer genetics in one way or the other. This is remarkable because his original, and sole, venture into the cancer problem was published nearly a century ago. Obviously, all of us striving to make significant contributions to science would be very pleased if our own work would be so prominently acknowledged at the beginning of the 22nd century. So the question is justified on why that is so. In trying to provide an answer I will, first, briefly summarize Boveri's contributions to cancer research and I do hope that I will be able to convey the esthetics of his thoughts and the clarity of the hypotheses of this remarkable cell biologist. Second, I will review what we know today of chromosomes and cancer and relate this knowledge to his previsions.

Most of Boveri's work was derived from analyses of see urchin and Ascaris eggs, for which he traveled to the Stazzione Zoologica in Naples, Italy. These studies resulted in the discovery of the centrosome as the coordinator of cell division. This alone can be considered a milestone, but Boveri also contributed early to the problem of cell polarity and the consequences for cellular differentiation. His student Spemann later received the Nobel Prize for his discovery of the organizer. His meticulous observations of the consequences of an abnormal number of centrosomes on cell division, which he induced by exposure to mechanical stress or through fertilization with two sperms led him to conclude that chromosomes should have both continuity in the interphase nucleus and, importantly, individuality, meaning that the information that one chromosome carries is different from another. These results were summarized in a series of publications during a period ranging from 1887 to 1909. He contributed to the problem of chromosome segregation errors using mathematical models to predict the probability with which different chromosome combinations would occur, and concluded that certain combinations would be incompatible with cell viability (for dedicated literature about his life and work the reader is referred to the following publications: [Stern, 1950; Baltzer, 1967; Cremer, 1985; Moritz and Sauer, 1996; Manchester, 1997; Neumann, 1998; Laubichler and Davidson, 2008; Maderspacher, 2008; Satzinger, 2008]).

At the turn of the century, Mendel's paper, first published in 1866, on the laws of heredity had been rediscovered. It was Boveri's seminal contribution that he immediately recognized that the postulated individuality of chromosomes could explain Mendel's laws if chromosomes would carry the genetic information: the chromosome theory of heredity was born (independently, Walter Sutton postulated the same [Sutton, 1903]). Now, what does this have to do with genomic instability and cancer? Boveri was a zoologist, and he recognized that. Hence, his humble apologies for entering the field of cancer research in his beautiful book "Zur Frage der Entstehung maligner Tumoren" which he published in 1914 [Boveri, 1914] (Fig. 1B). The English translation, by his wife and student Marcella Boveri, "The Origin of Malignant Tumors" appeared in 1929 [Boveri, 1929]. A recent, annotated translation by Henry Harris was published by Cold Spring Harbor Laboratory Press in 2008 [Boveri, 2008]. Boveri, however, rarely studied cancer tissues. But he projected his observation of dividing see urchin and Ascaris eggs and their abnormalities on what he perceived to be the genetic basis of malignancy. Others before him, most notably David von Hansemann, who had written "Über asymmetrische Zellteilung in Epithelkrebsen und

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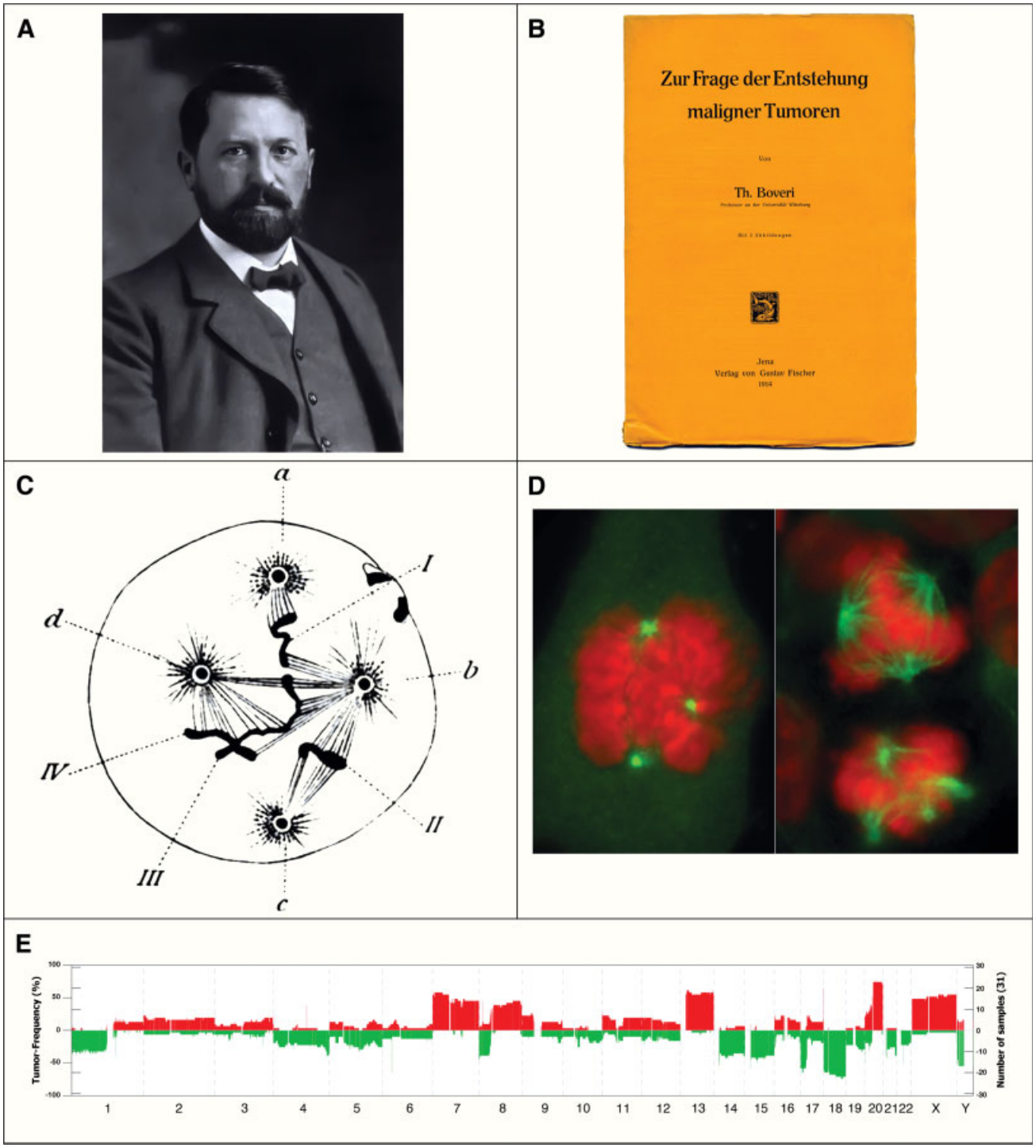

Fig. 1 (**A**) Theodor Boveri (1862–1915), pictured at age 46. (**B**) Title page of his seminal book (The origin of malignant tumors). (**C**) Drawing of an ascaris cell with four centrosomes [Boveri, 1888]. (**D**) Centrosome amplification in mouse cells deficient for the tumor suppressor gene *Brca1* (left panel, red stains DNA, γ-tubulin in green), and the tripolar and tetrapolar mitoses resulting from centrosome amplification in such cells (right panel, red stains DNA, α-tubulin in green). (**E**) The landscape of genomic imbalances in colorectal cancer. Red depicts chromosomal gains, while green indicates loss. Chromosomes and chromosome arms 7, 8q, 13q, and 20 represent tumor "promoting" chromosomes, while chromosome 14, 15, 17p, and 18 are "inhibiting" chromosomes. Note that chromosomes that are frequently gained are never lost, and vice versa, supporting the strong selective pressure to maintain these specific genomic imbalances. This plateau of genomic aberrations is maintained in metastases and in derived cell lines.

deren biologische Bedeutung" [Hansemann, 1890] (which can be translated as: On asymmetrical cell division in epithelial cancers and its biological relevance) had speculated on the role of chromosomes in tumorigenesis. Hansemann, for instance wrote: "Chromatin plays a crucial role for the inheritance of specific cellular features, and it is in particular the number of segments that carries important biological significance which is proven through the constant number in different tissues and species". This can only be interpreted as one of the first recognition that genetic material and its unequal distribution are correlated with tumorigenesis. As it often happens, the field was now ripe for more systematic exploration. Boveri was of course aware of Hansemann's publication, and refers to him in his own book. However, it was Boveri's transition from observations of multipolar mitoses in sea urchins to a chromosome theory of heredity and the theory of cancer as a disease of the chromosomes that makes his work so remarkably unique and different from that of other scientists.

In his book, Boveri postulated 20 specific hypotheses, summarized and paraphrased below, that are worth mentioning explicitly, because each of them addresses relevant questions in cancer biology and, maybe with one exception (hypothesis 16), have been verified through the study of cancer chromosomes during the almost 100 years that followed his publication, some of them only very recently:

1. A tumor cell carries an "irreparable defect" and this defect is located "not in the protoplasm but in the nucleus."
2. The uniform character of a tumor that persists in metastases and transplants would suggest that "typically every tumor arises from a single cell". His "tumor Anlage" could be paraphrased as "tumor stem cell".
3. Boveri argued that chromosomal aberrations in cancer cells result in disturbed metabolic activity with influence on the tumor environment.
4. For a tumor to arise from a certain tissue, specific chromosomal aberrations are required. However, he did not discard the possibility that several and different chromosome conditions could result in tumorigenesis, and that these differences would determine whether the tumor would be more or less similar to the original tissue. Highly de-differentiated tumors carry more cytogenetic abnormalities.
5. The differences between human and murine tumors can be explained because "the chromosomes, which in one form are independent, in the other have become associated."
6. Several similar or identical tumors can arise in the same organ.
7. To the problem of tumor inheritance Boveri writes that "there may be a hereditary transmission only in the sense that a certain disposition is transmitted," essentially suggesting recessive alleles. He continues that "this supposition takes for granted that in both of the parental germ cells, like chromosomes had the same abnormal condition." Hence, "inbreeding" would increase the risk of inheriting such conditions.
8. Tumors derived from the same organ can consist of different cells, which could be explained by different clones with different chromosomal abnormalities. This can occur at any stage of tumorigenesis.
9. Extending on the observation that double-fertilized sea urchin eggs with abnormal mitoses loosen tissue adherence Boveri hypothesized that a similar mechanism would be required for primary tumor cells to metastasize, and that this could have a genetic component as well.
10. Loosely paraphrased, Boveri speculates about the possibility of ongoing chromosomal instability that could lead to the additional genetic changes in the tumor.
11. Here he speculates that tissue differentiation could be partly attributable to not different chromosomes but activation or silencing of specific subchromosomal regions, and that the same feature could explain de-differentiation of tumors compared to the tissue of origin.
12. Tumorigenesis is promoted by injury or chronic inflammation, and aberrant cell division and subsequent tumorigenesis is more frequent on the basis of a tetraploid genome. This again was inferred from direct observation of sea urchin eggs.
13. In extension to the above-mentioned hypothesis he explains how radiation, certain chemicals, smoking, and chronic mechanical irritation can promote malignant transformation.
14. Parasites can promote tumorigenesis through inflammatory processes that lead—via increased proliferation—to the emergence of a benign papilloma. On the basis of the presence of this papilloma, which can be considered a bona fide premalignant lesion, tumor growth can occur when chromosome segregation errors have led to cells with tumor specific chromosomal aberrations. Once these are acquired, tumor growth is independent of the presence of the "parasite."
15. If certain parasites can cause tumors, "the carrying over of parasites to another individual exposes the latter to the same danger."
16. Boveri describes the rare circumstance that mice inoculated with a carcinoma develop sarcomas. He carefully, and somehow reluctantly, postulates that this could be due to the induction of aberrant mitoses as a consequence of "products" of the tumors.
17. Tumors occur more frequently in highly proliferative tissues, in particular if additional stimuli enhance proliferation.
18. He argues that despite the fact that tumorigenesis can have quite different causes, the same specific disturb-

ance in chromatin will eventually result. He continues and argues that "a tumor would result about as often as one would draw blindly a certain number out of a bag containing a thousand numbers" which led him to invoke a "lottery factor."

19. Here he speculates about the increasing incidence of carcinomas in the elderly and again explains this phenomenon with mitotic abnormalities that are more frequent in aged see urchin eggs.
20. In this final specific hypothesis, Boveri addresses the observation that nuclei of cancer cells are bigger than in normal cells, even though there were rare tumors for which smaller nuclei were described. First, he carefully again draws an analogy from see urchins, that is that nuclear size increases with the number of chromosomes. He then explains that while it is formally possible that tumors with less than normal numbers of chromosomes can occur, it is less likely than increased chromosome numbers because ensuing abnormal mitoses would remove chromosomes whose loss would not be compatible with cell survival.

The reader will have to agree that despite the fact that Boveri did not analyze tumor cells directly, his hypotheses of tumorigenesis were without exception correct. His "tumor Anlage" would be consistent with the concept of tumor stem cells and clonal evolution. The possibility of field cancerization was entertained, and he discussed familial predisposition to malignancy. The involvement of cell adhesion in metastasis and the role of chronic inflammation and radiation were recognized, and his hypotheses 14 and 15 would apply to HPV-induced cervical carcinogenesis. Hypothesis 20 is correct as well: most epithelial cancers have excess chromosomes, the exception being the rare category of chromophobe renal cell carcinomas, in which indeed specific chromosome losses dominate rendering these tumors hypodiploid [Speicher et al., 1994].

At this point I would like to expand to discuss, in the light of Boveri's predictions, what we have learned in recent years about the chromosomal composition of malignant tumors, with a particular emphasis on tumors of epithelial origin, i.e., carcinomas. In some recent publications about Boveri, it is often stated that his theory has been resurrected and his contribution to tumor genetics rediscovered. First, I am not sure to which extent his theory required resurrection nor whether his theories had ever been lost, because many older publications, periodicals and textbooks on cancer cytogenetics devote entire chapters to his model of chromosomal aberrations as a result of mitotic error [Koller, 1960, 1972; German, 1974]. Second, this "rediscovery" is probably mainly attributable to the fact that he postulated centrosome aberrations and resulting apolar mitoses as a cause of cancer. In 1996, Fukusawa et al. [1996] observed centrosome amplification and as a consequence of that abnormal mitoses in mouse embryonic fibroblasts deficient for the tumor suppressor gene p53. Soon after this intriguing observation, the cancer community's interest in centrosome abnormalities was rekindled. In fact, a Pubmed search using the terms "centrosome" and "cancer" produced 47 publications before 1996, but some 929 from then to the present. Subsequent reports confirmed the initial observation and recapitulated the findings for additional pertinent tumor suppressor genes, including *BRCA1*, *Rb*, *Ku70/80*, and *Gadd45a/a* [Hollander et al., 1999; Xu et al., 1999; Difilippantonio et al., 2000; Iovino et al., 2006], and established a correlation between centrosome abnormalities and overexpression of oncogenes, such as the Aurora kinase A and *PLK1*, the protein kinase *NEK2*, and cyclin A and cyclin E, among others [Zhou et al., 1998; Smits et al., 2000; Meraldi and Nigg, 2001]. In addition, centrosome abnormalities were observed in primary tumors and derived cell lines, and in some studies correlated with the degree of genomic instability (for reviews see, e.g., [Pihan and Doxsey, 1999; Schuyler and Pellman, 2001; Lingle et al., 2005; Nigg, 2006; Fukasawa, 2007]). In summary, there is now ample of evidence for a correlation of centrosome defects and cancer; however, their role in cancer initiation is far from clear. Considering the grossly aberrant mitoses that result from the amplification of functional centrosomes, it is not at all inconceivable that such cell divisions would produce progeny with a chromosome content incompatible with continued cell survival (see also the article by Difilippantonio et al. in this issue). (Boveri's drawing of a cell with four centrosomes and an example of centrosome amplification and—as a consequence—tripolar mitoses in BRCA1-deficient cells are shown in Figs. 1C and 1D).

But that is precisely not what Boveri postulated. As a matter of fact, he suggested that as a consequence of abnormal mitoses in most instances cell death would ensue; he merely considered abnormal centrosome numbers as a means for chromosome segregation errors to occur. He wrote: "Inasmuch as my theory demands a certain wrong arrangement of the chromatin-complex as a condition for the origin of malignant tumors, (this) cannot only be obtained in different ways, but is also in its origin dependent on chance to a high degree. . .." Boveri proposes that the cause of cancer is an imbalance of specific chromosomes, consistent with the presence of "an irreparable defect" which is "located not in the protoplasm but in the nucleus." He becomes even more specific in one of the most beautiful passages in his book, which reads: "To assume the presence of definitive chromosomes which inhibit division, would harmonize best with my fundamental idea. If their inhibitory action were temporarily overcome by external stimuli, the cell-division would follow. Cells of tumors with unlimited growth would arise if those 'inhibiting chromosomes' were eliminated." And he con-

tinues: "On the other hand, the assumption of the existence of chromosomes which promote division, might satisfy this postulate. On this assumption, cell-division would take place when the action of these chromatin parts, which are as a rule too weak, should be strengthened by a stimulus; and the unlimited tendency to rapid proliferation in malignant tumors cells would be deduced from a permanent predominance of the chromosomes that promote division." One can be tempted to reduce his predictions as describing the concept of tumor suppressor genes, residing on the "inhibiting chromosomes" and oncogenes, located on the promoting chromosomes. All this at a time when the nucleus and with it the chromosomes were not generally accepted as the carrier of genetic information!

However, his concept was even more accurate than it appears at first glance and I am inclined to state that his conclusions have only recently become appreciated by the cancer cytogenetic community (which would be those studying cancer chromosomes), and not to the fullest extent yet by the cancer genetic community (referring to our colleagues concentrating their efforts on specific cancer genes or pathways).

To support this assessment, I will briefly review our knowledge about chromosomal aberrations in epithelial cancer that mostly emerged with the application of molecular cytogenetic techniques to the study of the genomes of carcinomas. The paradigm of translocation induced oncogene activation in hematological malignancies has long been established. The detection of the Philadelphia chromosome in patients with chronic myelogenous leukemia [Nowell and Hungerford, 1960; Rowley, 1973] and a recurrent *MYC*-activating translocation in Burkitt's lymphoma cells [Manolov and Manolova, 1972; Zech et al., 1976] provided the first evidence that cancer is a disease of the chromosome. The application of chromosome banding techniques to study tumors of epithelial origin was by far more cumbersome [Heim and Mitelman, 1995]. The difficulty to culture carcinomas, poor chromosome morphology, and the sheer number of chromosomal aberrations, some exceedingly complex, made a comprehensive cytogenetic analysis often very difficult if not impossible. All this led to the perception that karyotypes of tumors of epithelial origin would be governed by cytogenetic chaos, brought upon by catastrophic mitoses as a consequence of centrosome aberrations and telomere dysfunction and maintained by genomic instability. With the advent of molecular cytogenetic techniques, and in particular the development of comparative genomic hybridization [du Manoir et al., 1993; Kallioniemi et al., 1992] and spectral karyotyping (SKY) [Schröck et al., 1996] or M-FISH [Speicher et al., 1996] that allowed to comprehensively survey cancer genomes this perception needed to be revisited [Ried et al., 1997, 1999]. Several facts emerged: the genomes of carcinomas are defined by a recurrent pattern of chromosomal gains and losses, which translates to a landscape of genomic imbalances that is strictly conserved. Second, balanced chromosomal aberrations are rare which means that translocation-induced oncogene activation, the hallmark of hematological malignancies is not prevalent in carcinomas. Furthermore, chromosomal aneuploidies, which are the cause of the observed genomic imbalances, are acquired at early stages during tumorigenesis when the genome can still be stable (in cervical tumorigenesis already in low-grade dysplastic lesions, in the colorectal cancer sequence in small polyps). These early imbalances are maintained in stages of invasive and metastatic disease. The probability for the acquisition of these imbalances is increased by chronic inflammation, as demonstrated by the considerable risk for the development of colorectal cancer in patients with inflammatory bowel disease and for esophageal cancer in Barrett's esophagus (Boveri's hypothesis 12), or by the association of infection with human papillomavirus (Boveri's "parasite") and cervical cancer. But what we and many others have observed would also be consistent with the "lottery" that Boveri invoked, i.e., a stochastic chromosome segregation error. We believe that the process of chromosome segregation, while under strict surveillance, is not infallible. As much as mutations can occur during DNA replication despite proofreading, one daughter cell can at some point in time receive three chromatids and its sibling only one even in the presence of intact mitotic cell cycle checkpoints. And, yet another of Boveri's correct predictions, i.e., that a tetraploidization of the genome would increase the possibility for such a segregation error to occur is entirely consistent with the aberration patterns that we observe during cervical carcinogenesis using interphase cytogenetics with specific DNA probes [Heselmeyer-Haddad et al., 2005]. Frequently, but not necessarily, can one observe aberration patterns that indicate that the acquisition of extra copies of chromosome 3 was preceded by a tetraploidization of the genome. It is in our view entirely reasonable to argue that the distribution of twice the number of chromosomes increases the likelihood that an error can occur during mitotic cell division, as much as a juggler's probability of dropping a ball increases with the number of balls. If it is an error affecting the "right" chromosome in a tissue environment in which it is critical, increased proliferation and clonal expansion would follow, which in turn increases the propensity for the acquisition of additional aberrations, including additional chromosomal gains and losses, required for the progression to invasive and metastatic disease. And this is exactly the cytogenetic profile observed in epithelial cancer. For instance, essentially all cervical carcinomas have a gain of chromosome 3q [Heselmeyer et al., 1996]. This specific imbalance in cervical cancer is as common as the Philadelphia chromosome is in CML and it is therefore justified to consider this particular genome mutation as a fundamental event

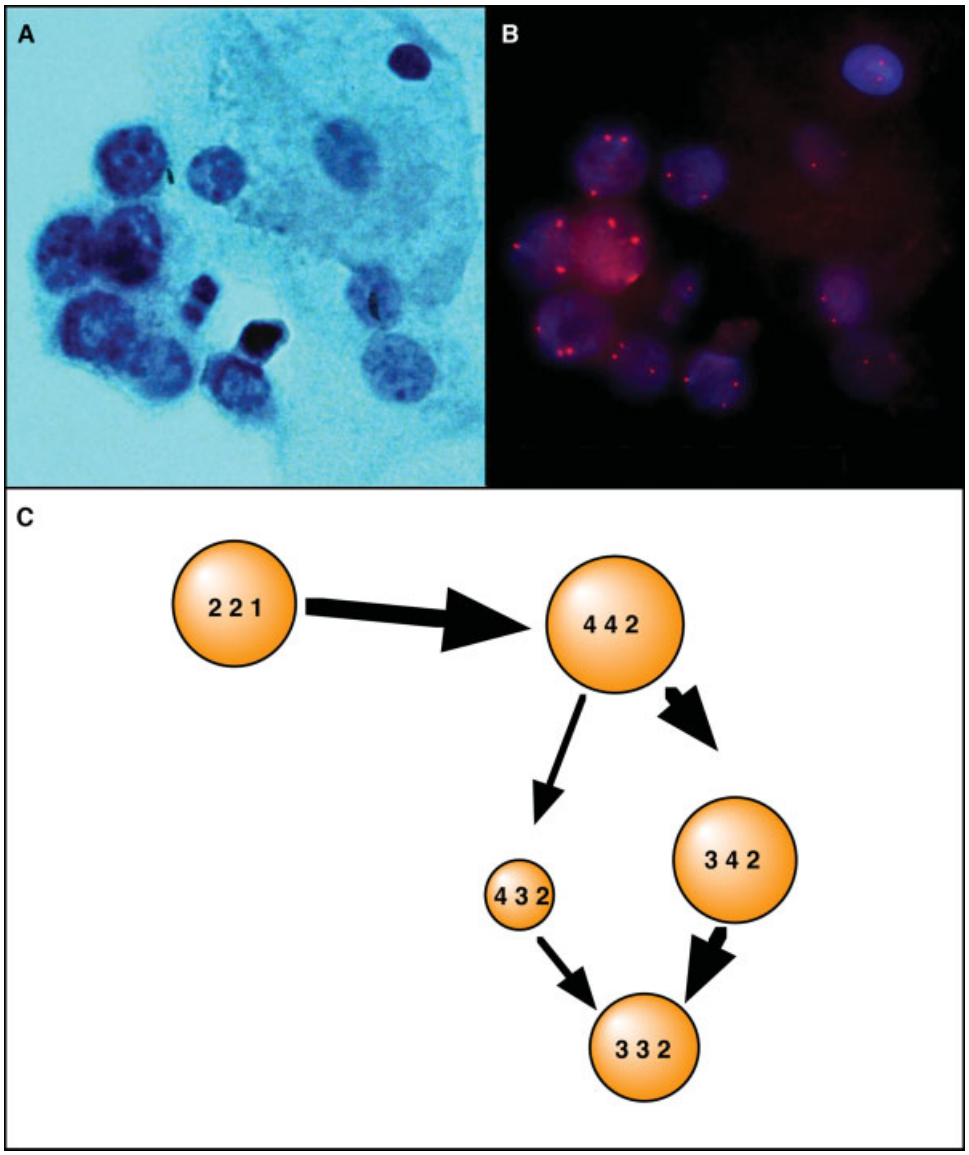

Fig. 2(**A**) Examples of the emergence of chromosomal aneuploidy during cervical cancer development. (A) Cells after Pap-staining. (**B**) FISH with a probe for chromosome 3 (red dots) in the same cells shown in (A). This picture supports several of Boveri's hypotheses: the same clonal pattern of three copies of a "promoting" chromosome in the adjacent cells in this cytological sample strongly suggest a clonal evolution event originating from a single cell. These cells are HPV-positive (Boveri's "parasite"), and cervical cancer is, of course, a sexually transmitted infectious disease (hypothesis 15). (B) The dynamic of chromosomal changes in breast cancer. Clonal patterns of chromosomal aneuploidy in direct touch preparations. The first number refers to copy numbers of chromosome 10, the second to chromosome 1, and the third to chromosome arm 17p (targeting *TP53*). The initial event would be a loss of the short arm of chromosome 17 in a diploid cell (pattern 221), which leads to the tetraploidization of the genome (pattern 442). Random chromosomal gains and losses can occur, but, of note, the initial loss of the "inhibiting" chromosome (17p), as Boveri postulated, remains present in more than 95% of cells in this tumor.

for tumorigenesis. These interpretations are supported by the experiment depicted in Figures 2A and 2B, which lends convincing support to several of Boveri's specific hypotheses. In this experiment, we have used fluorescence in situ hybridization with a probe specific for chromosome 3 and detected three copies of this chromosome in a cluster of cells in this cytological specimen (Pap smear). Lesions with this chromosome constitution progress to invasive disease, and the gain of chromosome 3q is maintained (hypothesis 1, a "irreparable defect in the nucleus"). The fact that all these cells carry the identical clonal pattern of aneuploidy is entirely consistent with it having originated from a "single cell" (hypothesis 2). The gain of chromosome 3 is specific for cervical cancer, i.e., other chromosomes are not relevant in this tissue context, which he describes in hypothesis 4 ("It is conceivable that there is for a definite kind of cell only a single abnormal chromosome combination which gives the cell

the quality of malignity, whereas all other combinations would be either harmless or would produce cells unable to live"). The development of cervical cancer, as we know, requires infection with high-risk HPV [zur Hausen, 2002], consistent with Boveri's "parasite" suggested in hypothesis 14, which makes cervical cancer a sexually transmitted infectious condition (hypothesis 15). Finally, cervical cancers often arise after the tetraploidization of the genome (hypothesis 12), and essentially always carry more chromosomes than normal cells (hypothesis 20).

In colorectal carcinomas the situation is similar, however, different chromosomes are involved: while chromosome 3 plays no role in this organ, gains of chromosomes and chromosome arms 7, 8q, 13, and 20q, accompanied by losses of chromosome arms 17p and 18q are invariably observed in sporadic colorectal carcinomas, emerging in preinvasive dysplastic lesions [Bardi et al., 1993; Ried et al., 1996] (see also Fig. 1E). Even after years of propagation under tissue culture conditions, a cell line derived from a colorectal carcinoma would be recognized as such based solely on its signature of genomic imbalances [Ghadimi et al., 2000; Kleivi et al., 2004; Camps et al., in press]. In other words, cancer genomes are not randomly scrambled but arrive at a certain, yet stable plateau of tumor specific, and to a certain extent, tumor stage specific genomic imbalances. Continued proliferation of malignant cells apparently requires the loss and gain of specific "inhibiting" and "promoting" chromosomes. This again is supported by the results shown in Figure 2C. Here, FISH with a three-color panel specific for chromosomes 1, 10, and for the short arm of chromosome 17 (targeting *TP53*) was performed on direct touch preparations from a breast cancer. More than 95% of the cells in this preparation show copy number reduction of chromosome arm 17p. This loss is maintained despite the fact that chromosomes that do not carry an "inhibiting" function in breast cancer can be randomly lost as a reflection of chromosomal instability. What we observe here is a fundamental biological requirement whose importance is further supported by the observation that those chromosomes recurrently gained in a specific tumor are very rarely, if not at all, lost, and those commonly lost are never regained (Fig. 1E). In other words, the distribution of these imbalances must be driven by a strong, almost insurmountable, continuous selection for the maintenance of certain chromosomal gains and losses. This strong conservation triggers the question regarding the teleology of these aneuploidies, in particular as it relates to the consequences on the transcriptome of cancer cells. One could postulate that the reason for the recurrent gain of a specific chromosome is a copy number increase for an oncogene or for several oncogenes that reside on said chromosome. Genes other than these targets would therefore not be transcriptionally active. Conversely, one could postulate that the consequence of increased genomic copy number is a proportional increase in message for most or all genes on a given chromosome. The verdict is now in: multiple studies have established a positive correlation of genomic copy number and transcript levels in primary tumors, cell lines, and models constructed to specifically address this question. It is therefore justified to state that genomic aneuploidies in carcinomas result in a massive deregulation of the transcriptional equilibrium through the aneuploidy-dependent up or downregulation of thousands of genes [Pollack et al., 2002; Upender et al., 2004; Wolf et al., 2004; Grade et al., 2006]. Neve et al. [2006] assessed the functional space of genes deregulated by low-level copy number changes and found that many of them belong to genes involved in cellular metabolism. One could therefore speculate that the activation of specific oncogenes, and the inactivation of tumor suppressor genes act in concert with the deregulation of genes as a consequence of low-level copy number changes that provide the metabolic infrastructure for increased proliferation. One of the challenges in understanding the genome mutations in carcinomas will be to elucidate whether the presence of a tumor suppressor gene on frequently lost chromosomes, or the presence of an oncogene on frequently gained chromosomes is sufficient to fully explain the reason for the defining and recurrent patterns of genomic imbalances. In other words, we will need means to experimentally dissect the relative contribution of specific oncogene activation vis-a-vis the global transcriptional deregulation imposed by chromosome-wide copy number changes. Only then will we be in a position to truly verify or falsify Boveri's central statement, i.e., the dominant role of inhibiting and promoting chromosomes that formed the basis for his chromosome theory of cancer.

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